THE JOHNS HOPKINS UNIVERSITY

BALTIMORE, MARYLAND 21218

DEPARTMENT OF BIOLOGY

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SENT VIA FAX

Ru Chih Huang, Ph.D.
Institute of Molecular Biology
Academia Sinica
Nankang, Taipei, 11529
REPUBLIC OF CHINA

Dear Ru Chih:

I noticed in my letter from Dr. Y. H. Tseng, Director Agricultural Biotechnology Laboratories at Taichung that he would like to have an abstract of the keynote talk that he asked me to give at the beginning of his symposium on the morning of January 12. I thought you might be able to transmit this to him if I send it to you by your office FAX machine, and then someone from his laboratories could pick it up. He seems to be in somewhat of a hurry to get the abstracts, so I thought I'd better do it this way. The abstract follows:

> Since I am neither a fisherman nor a farmer, my presentation to you will have to be based entirely on the kinds of protein chemistry to which I have been exposed. Protein molecules from eukaryotic organisms when synthesized from cloned information that has been inserted into a bacterial host cell must often be further processed before it reaches a state of functional significance. A most common problem involves sulfhydryl groups and their conversion to SS bonds. When several SH groups are present there is, of course, the problem of mismatching so that the wrong tertiary structure is achieved and frequently a mixture of several possible conformations. There is also the problem of processes such as glycosylation which are post-translational modifications monitored by specific enzymes mainly lacking in bacterial organisms. We considered the problem of disulfide bond formation some time ago when it was found that a molecule such as bovine pancreatic ribonuclease tended to form upon folding a very large number of incorrect structures because of improper disulfide bond formation. At that time we discovered in rat liver an enzyme, now called protein disulfide isomerase, which rearranges disulfide bonds and, presumably due

to the pressure of attaining the thermodynamically most stable structure, eventually converts incorrect S-S bonds to correct ones. This enzyme turned out to be most commonly found and in largest quantities in those tissues of higher organisms that are involved in active secretion of large amounts of protein material. This enzyme is lacking in bacteria, but there is a substitute, thioredoxin, which is present in E. coli, for example, that can carry out a similar function. Thioredoxin which contains one disulfide bond interacts with NADH and the S-S bond is inverted to sulfhydryl groups. These groups are able to function like the isomerase that we employed and, presumably, is available within the bacterial cell to "correct" newly synthesized but improperly folded protein products. I can say nothing about the problem of glycosylation or of other post-translational modifications although some of you here may be aware of enzyme systems in bacteria that can monitor these kinds of processes.

In addition to some consideration of the folding problem, I intend to make a few remarks about what is called "the motility" of proteins in general and the effects of substrates and inhibitors on the stabilization of three-dimensional structure. I will make a few remarks as well about the rate of protein folding and the importance of various kinds of local nucleation of short stretches of the peptide chain that might account for the high rate of formation of functional protein molecules.

Sincerely,

Chris Anfinsen

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